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13. ABSTRACT ***(Maximum 200 Words)***
We are developing methods to image her-2-neu oncogene over-expression in breast cancer using positron emission tomography (PET). Small oligodeoxynucleotides (ODNs) that are complementary to the Her-2-neu messenger RNA (mRNA) are being investigated as potential imaging probes. Fluorine-18 (2 hour half-life positron emitter) has been used to label 15-18 mer ODN probes. The labeling of an ODN to Fluorine-18 has been particularly troublesome because of the limited half-life and the complicated chemistry. We have explored multiple strategies and are trying to maximize yield and specific activity of our probes. With adequate synthesis of the ODN probes we will begin further cell testing and small animal imaging with microPET. We expect that the techniques developed will lead to methods to detect breast cancer in living subjects in the case that her-2-neu is over-expressed.

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Table of Contents

Introduction

Improved methods to image breast cancer are critically needed in order to lead to earlier initial detection, earlier detection for recurrence, and better management of patients undergoing treatment. Most approaches to date have focused on anatomical changes due to tumor growth (e.g., mammography, computerized tomography, magnetic resonance imaging) or metabolic changes in the tumor (e.g., FDG Positron emission tomography). As molecular oncology continues to shed insight into the molecular basis for breast cancer, methods are needed to directly image molecular abberations in breast cancer cells. We are developing methods using radiolabeled antisense oligodoeoxynucleotides (RASONs) which can be injected via the blood-stream and then accumulate in cells that have sufficient levels of a particular target messenger RNA (mRNA). Normal cells (breast and other tissues) which don't have high level of target mRNA would not lead to intracellular trapping of the RASONs. One known molecular abnormality in about 25% of breast cancer patients is the over-expression of the Her-2-neu (c-erb-B2) oncogene. We have selected this gene as our first target using RASONs labeled with fluorine-18 (a positron emitter). We seek to develop RASONs that can be validated using nude mice carrying human breast cancer tumor xenografts imaged using microPET technology. With pre-clinical proof of their ability to home to breast cancer tumors over-expressing Her-2-neu we hope to have sufficient proof to eventually transition to human applications. It is hoped that this approach will lead to more specific and sensitive detection of breast cancer with over-expression of Her-2-neu and set the foundation for a new antisense based imaging approach which could potentially be applied to many different oncogenes.

Body

Aim 1: The development of ^{18}F-labeled oligodeoxynucleotides.

After several unsuccessful attempts we have developed a general and efficient method for the synthesis of ^{18}F-labeled oligodeoxynucleotides (ODNs). Following is a briefly summary of our findings and accomplishments during the past 3 years.

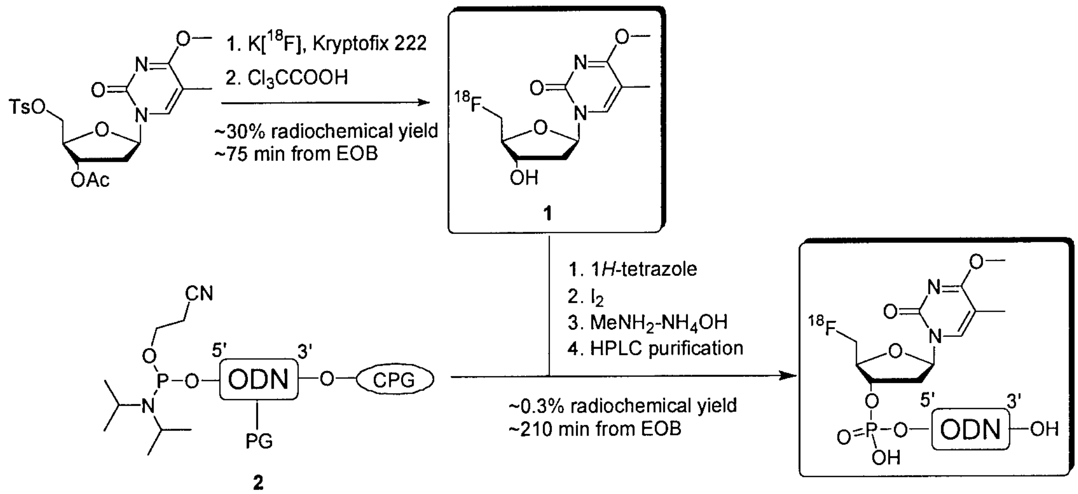
1.1. Unsuccessful attempts.

1.1.1. Direct coupling of an [^{18}F]fluorinated nucleoside with a CPG-bound 5'-oligodeoxynucleotide phosphoramidite (CPG = controlled pore glass resin). We initially planned to synthesize [^{18}F]fluorinated ODNs by employing the reverse-activation protocol, which was successfully employed to synthesize ^{14}C- and ^{3}H-labeled ODNs using an automated ODN synthesizer.[1] Thus, we synthesized 5'-deoxy-5'-[^{18}F]fluoro-4-*O*-methylthymidine (**1**) and coupled with a CPG-bound 5'-ODN phosphoramidite **2** according to the reverse-activation protocol (Scheme 1). Although the synthesis of **1** was successful [~30% decay-corrected radiochemical yield with >99% radiochemical purity within ~75 min from end of bombardment (EOB)], the subsequent coupling reaction was found unsatisfactory due to its poor radiochemical yield (~0.3% decay corrected) and low specific radioactivity [~1.3 mCi/μmol at end of synthesis (EOS)]. Systematic investigations revealed critical problems associated with this approach, including low coupling efficiency of the reverse-activation method with a trace amount of **1** (a typical condition of ^{18}F-chemistry) and partial de[^{18}F]fluorination under the standard ammonolytic deprotection and cleavage condition (Scheme 2), resulting in difficult purification of the ^{18}F-labeled ODN.

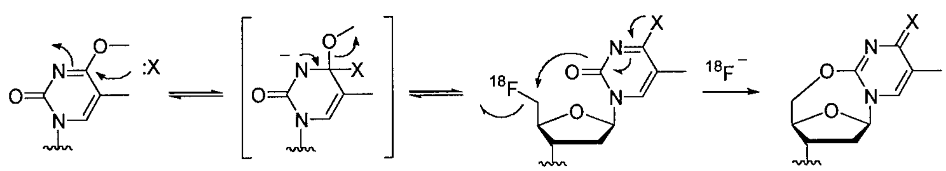
1.1.2. Reductive alkylation of an amino-functionalized oligodeoxynucleotide with 4-[^{18}F]fluorobenzaldehyde. The reductive alkylation of amines with aldehyde derivatives is a common practice in the modification of biomolecules including ODNs and proteins.[2] Therefore, we investigated the reductive alkylation of a 5'-aminohexyl ODN **3** with readily available 4-[^{18}F]fluorobenzaldehyde[3] (**4**) (Scheme 3). Although a model experiment using non-radioactive 4-fluorobenzaldehyde was successful, radiochemical synthesis with **4** yielded only 4-[^{18}F]fluorobenzyl alcohol (**5**) (a reduction product of **4**) as a sole product. Various conditions were examined without any success. We concluded that **4** was reduced by reducing reagents (e.g., $NaBH_4CN$) before the

formation of the imine intermediate under the typical condition of ^{18}F-chemistry where reducing reagents were present in large excess over **4**.

Scheme 1. Direct coupling of [^{18}F]fluorinated nucleoside 1 with CPG-bound 5'-ODN phosphoramidite 2.

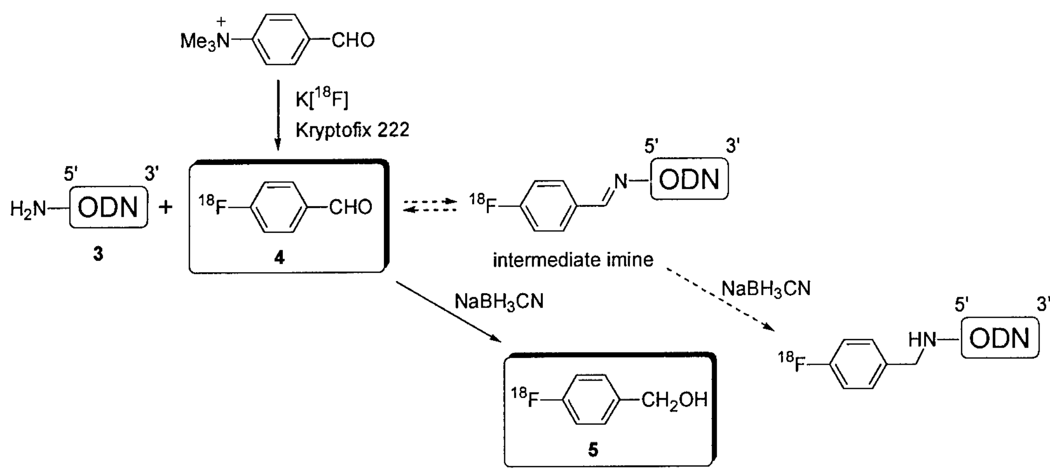


Scheme 2. A possible mechanism of de[^{18}F]fluorination under the standard ammonolytic deprotection and cleavage condition.



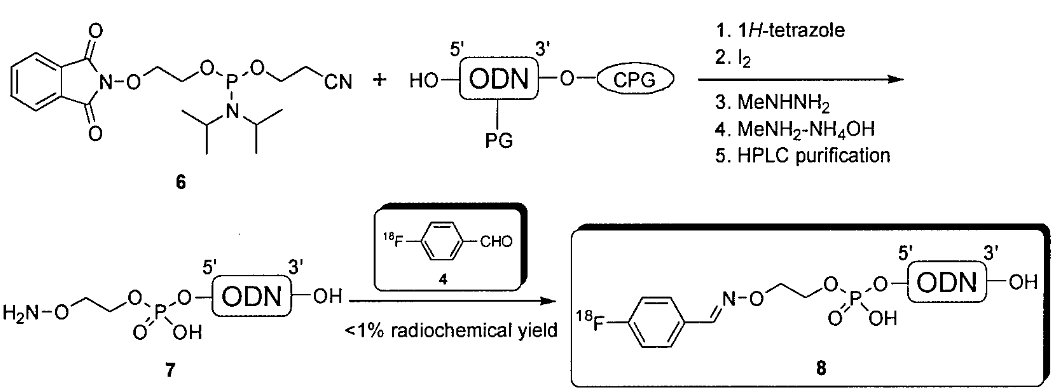
X = $MeNH_2$, NH_3, OH^-

Scheme 3. Reductive alkylation of amino-functionalized ODN 3 with 4-[^{18}F]fluorobenzaldehyde 4.

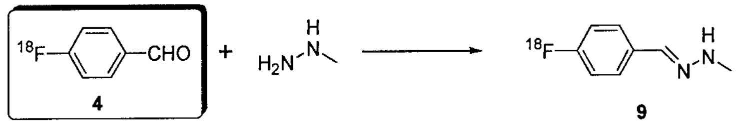


1.1.3. Aminooxy-aldehyde coupling of an aminooxy-functionalized oligodeoxynucleotide with 4-[^{18}F]fluorobenzaldehyde. The aminooxy group is more nucleophilic than the primary amino group because of the "α-effect".[4] In addition, the *O*-alkyl oximes formed upon the reaction of *O*-alkylhydroxylamines with aldehydes are much more stable than the imines derived from primary amines, thus eliminating the use of reducing agents.[4] It was therefore anticipated that the aminooxy-aldehyde coupling reaction would eliminate the problem associated with the reductive alkylation approach. We first synthesized the base-labile phthalimide-protected aminooxy modifier **6** and incorporated into an ODN assembled on the CPG support using an automated ODN synthesizer (Scheme 4). After the sequential treatment with $MeNHNH_2$ (for removal of the phthalimide group) and with $MeNH_2$-NH_4OH (deprotection of other base-labile protecting groups and cleavage of the synthesized ODN from the CPG support), the corresponding aminooxy-functionalized ODN **7** was obtained. The oxime formation between **7** and **4** was however not satisfactory yielding the desired [^{18}F]fluorinated ODN **8** only in less than 1% radiochemical yield. The major product was identified to be *N*-(4-[^{18}F]fluorobenzylidene)-*N*-methylhydrazine (**9**), which was formed by the reaction of **4** and the residual $MeNHNH_2$ (Scheme 5). To our surprise, $MeNHNH_2$ was still present even after purification with reversed-phase HPLC. We therefore decided to use the acid-labile monomethoxytrityl (MMT) protecting group in the place of the phthalimide group. Thus, MMT-protected aminooxy modifier **10** was prepared and used to assemble the MMT-protected ODNs on the CPG support (Scheme 6). The standard ammonolytic deprotection and cleavage yielded the corresponding MMT-protected ODN **11**. Contrary to our expectation, deprotection of the MMT group under acidic conditions was found problematic due to the ensuing decomposition of the parent ODN. A similar problem during the MMT deprotection of ODNs has recently been reported in the literature.[5]

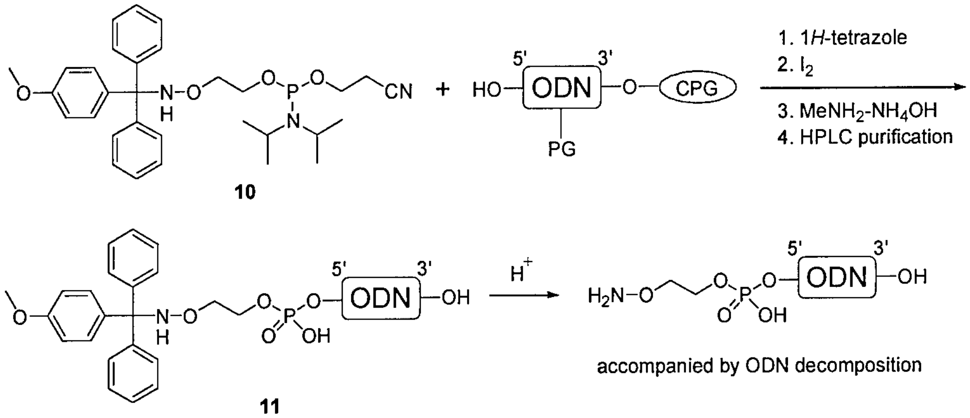
Scheme 4. Aminooxy-aldehyde coupling of aminooxy-functionalized ODN 7 with 4-[^{18}F]fluorobenzaldehyde 4.



Scheme 5. Formation of *N*-(4-[^{18}F]fluorobenzylidene)-*N*-methylhydrazine 9.

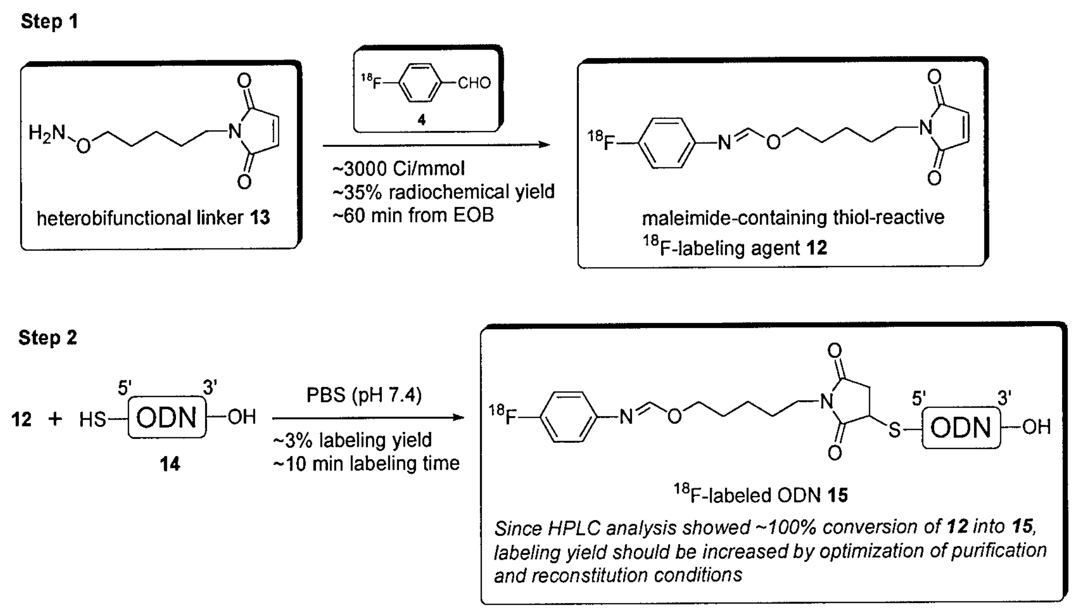


Scheme 6. Attempted synthesis of aminooxy-functionalized ODN using MMT-protected aminooxy modifier 10.



1.2. Successful synthesis of an ^{18}F-labeled ODN: an efficient and general approach to ^{18}F-labeling of oligodeoxynucleotides and their synthetic analogues. After unsuccessful attempts summarized above, we have finally succeeded in the synthesis of an ^{18}F-labeled ODN with the aid of a new maleimide-containing thiol-reactive ^{18}F-labeling agent, *N*-{4-[(4-[^{18}F]fluorobenzylidene)aminooxy]butyl}maleimide (**12**) (Scheme 7). We have synthesized **12** by reacting **4** with a new heterobifunctional linker, *N*-[4-(aminooxy)butyl]maleimide (**13**), which contains a thiol-reactive maleimide group and an aldehyde-reactive aminooxy group. The ^{18}F-labeling agent **12** (~3000 Ci/mmol at EOS) was obtained in ~35% decay-corrected radiochemical yield within ~60 min from EOB. Treatment of the 5'-end thiol-functionalized ODN **14** with **12** (~30 mCi) in PBS (pH 7.4) at room temperature for ~10 min afforded, after purification and reconstitution, chemically and radiochemically pure ^{18}F-labeled ODN **15** (~1 mCi) in PBS (1 mL). Monitoring this labeling process by HPLC showed that the radioactivity of **12** was almost completely incorporated into the ODN within 10 min, indicating the high efficiency of **12** as a thiol-reactive ^{18}F-labeling agent. We are currently optimizing the conditions for purification and reconstitution of the ^{18}F-labeled ODN. Upon the establishment of these conditions, ^{18}F-labled ODNs will become available on a routine basis for *in vivo* evaluation.

Scheme 7. Successful synthesis of ^{18}F-labeled ODN.



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<u>**Aim 2:**</u> **The development of (15-20)-mer oligodeoxynucleotides for targeting the Her-2-neu (c-erbB-2) proto-oncogene mRNA.** We have studied several candidate sequences for targeting the Her-2-neu mRNA. Through structural analysis we have previoiusly defined several optimal sequence that we feel should be accessible by our RASON probes. We have completed synthesis of modified-backbone ODNs in order to improve their plasma stability. We find that 2' O-methyl modified ODNs may be

optimal for eventual use *in vivo*. We are also currently exploring 2' methoxy-ethoxy modified ODNs as potential probes. We continue to collaborate with scientists at ISIS Pharmaceuticals (Carlsbad, CA) which is a company that specializes in ODN therapeutics. We are hopeful that as our labeling chemistry is optimized, we can test many new generation of ODNs for eventual *in vivo* use.

Aim 3: **Tissue culture testing of the developed probes to determine the specificity and kinetics of the probe for the c-erbB-2 mRNA.** We have studied 4 cell lines for their levels of Her-2-neu expression. These include a MCF-7 control cell line, a MCF-7 over expressing Her-2-neu, SK-BR-3, and SK-OV-3. We now await the labeling of this ODN sequence with Fluorine-18 after Aim 1 leads to improved yields for our RASON probes. Then cell culture uptake and efflux studies will be performed with the RASON probes (antisense and control probes).

Aim 4: **To study the targeting properties of ^{18}F-labeled antisense-oligodeoxynucleotides *in vivo* in a mouse animal model using PET.**

There has been no progress towards this aim as compared to the previous report. We still await further progress of Aim 1 to go further in Aim 4. We have performed some very preliminary studies in two control nude mice in order to understand the limitations of injecting our 18F-ODN probes into mice and imaging with a microPET. Because the yields of 18F-ODN are still very low (see also Aim 1), we have not been able to get satisfactory images of biodistribution of the tracer. We will be able to better characterize the biodistribution when more tracer is routinely available. We have also been able to grow xenografted tumors in mice (e.g., MCF-7) in order to eventually use these tumor models to image with microPET and our 18F-ODN probes.

Key Research Accomplishments

- Synthesis of 18F-oligodeoxynucleotide (ODN) probes in low yields
- Multiple strategies for synthesis thoroughly explored over the last 3 years
- Purification of 18F-ODN probes for cell culture testing and *in vivo* testing
- Assessment of hybridization potential of 18F-ODN with target mRNA through T_m measurements
- Synthesis of 2'-o-methyl modified ODNs for improved plasma stability
- Specific Activity measurements of 18F-ODN probes
- Isolation of an 18-mer antisense sequence that should have optimal targeting properties for Her-2-neu
- Study of cell lines for levels of Her-2-neu over-expression
- Preliminary biodistribution studies of 18F-ODN probes in control mice using microPET

Reportable Outcomes

Publications

D. Pan, **S.S. Gambhir**, T. Toyokuni, M. Iyer, N. Acharya, M.E. Phelps, J. Barrio. Rapid Synthesis of a 5'-Fluorinated Oligodeoxynucleotide: A Model Antisense Probe for use in Imaging with Positron Emission Tomography (PET). Bioorganic & Medicinal Chemistry Letters, 8(11):1317-1320, 1998.

Abstracts

D. Pan, T. Toyokuni, J.R. Barrio, N. Satyamurthy, M.E. Phelps, **S.S. Gambhir**. Synthesis of a Fluorine-18 Labeled Antisense Oligodeoxynucleotide as a Probe for Imaging Gene Expression. Journal of Nuclear Medicine, 40(5):82P, 1999.

J.C. Walsh, K.M. Akhoon, N.Satyamurthy, J.R. Barrio, M.E. Phelps, **S.S. Gambhir**, T.Toyokuni. Application of Silicon-Fluoride Chemistry to Fluorine-18 Labeling Agents for Biomolecules: A Preliminary Note. Presented at the 13th International Symposium on Radiopharmaceutical Chemistry, St. Louis, Missouri. July, 1999.

J.C. Walsh, D. Pan, N. Satyamurthy, J.R. Barrio, M.E. Phelps, T. Toyokuni**, S.S. Gambhir**. Use of 5'Deoxy-[18F] Fluoro-4-0-Methylthymidine in the Synthesis of 18F-Labeled Antisense Oligodeoxynucleotide Propbes for Imaging Gene Expression with PET. Journal of Nuclear Medicine, 41(5):245P, 2000.

J.C. Walsh, L.M. Fleming, N. Satyamurthy, J.R. Barrio, M.E. Phelps, **S.S. Gambhir**, T. Toyokuni. Application of Silicon-Fluoride Chemistry for the Development of Amine-Reactive 18F-Labeling Agents for Biomolecules. Journal of Nuclear Medicine, 41(5):249P, 2000.

T. Toyokuni, J. C. Walsh, R. J. Abdel-Jalil, A. Dominguez, J. E. Shively, N. Satyamurthy, J. R. Barrio, M. E. Phelps, A. M. Wu, and **S. S. Gambhir**. Synthesis of a New Maleimide-Containing Thiol-Reactive ^{18}F-Labeling Agent, *N*-{4-[(4[^{18}F] Fluorobenzylidene)Aminooxy]Butyl} Maleimide, and it's Application to the Labeling of an Oligodeoxynucleotides. Journal of Nuclear Medicine, 42(5):256P, 2001.

R. J. Abdel-Jalil, J. C. Walsh, J. L. Stone, J. E. Shively, N. Satyamurthy, J. R. Barrio, M. E. Phelps, A. M. Wu, **S. S. Gambhir**, and T. Toyokuni. Synthesis of 4-[(4-^{18}FFluorobenzylidene) Aminooxy]Butyl Vinyl Sulfone as an Amine- and Thiol-Reactive ^{18}F-Labeling Agent. Journal of Nuclear Medicine, 42(5):256P, 2001.

Conclusions

The results to date demonstrate that it is possible to label oligodexoynucleotide molecules with Fluorine-18 (a positron emitter). We still continue to optimize the chemistry in order to achieve significant yields at a high specific activity. Many of the other Aims are ready to proceed once we have sufficient F-18 labeled ODNs. These include study of cell culture models and *in vivo* animal tumor models using microPET imaging technology. The groundwork has also been set for further study in cell culture models, and *in vivo* animal models.

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D. Pan, **S.S. Gambhir**, T. Toyokuni, M. Iyer, N. Acharya, M.E. Phelps, J. Barrio. Rapid Synthesis of a 5'-Fluorinated Oligodeoxynucleotide: A Model Antisense Probe for use in Imaging with Positron Emission Tomography (PET). Bioorganic & Medicinal Chemistry Letters, 8(11):1317-1320, 1998.

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Appendices

Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1317–1320

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

RAPID SYNTHESIS OF A 5′-FLUORINATED OLIGODEOXY-NUCLEOTIDE: A MODEL ANTISENSE PROBE FOR USE IN IMAGING WITH POSITRON EMISSION TOMOGRAPHY (PET)[1]

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Abstract: 5′-Deoxy-5′-fluoro-O^4-methylthymidine was synthesized by the reaction of the corresponding 5′-*O*-tosylate with KF in the presence of Kryptofix [222] and coupled to a 5′-phosphoramidite-activated CPG-bound oligodeoxynucleotide. The sequence of reactions and purifications were accomplished within 4 h, a necessary condition of the development of radiofluorinated antisense oligodeoxynucleotide probe for use with PET.
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Recent years have seen dramatic developments in the application of synthetic antisense oligodeoxynucleotides (ODNs) as inhibitors of specific disease-related gene expression.[2,3] The antisense approach has also preliminarily been explored to develop new biological probes for in vivo imaging of specific gene expression. Gamma-emitting ^{111}In- and ^{99}mTc-labeled antisense ODNs have been recently synthesized for use with single photon emission computed tomography (SPECT) imaging.[4] However, labeling with these radioactive metals requires a sterically bulky chelating group that might alter the binding affinity as well as cellular transport and distribution of the parent ODNs. In addition, SPECT has a lower resolution than positron emission tomography (PET) (8–12 mm vs. 2–6 mm, respectively). Furthermore, as compared with SPECT, PET allows for greater quantitative accuracy that is essential for developing a quantitative in vivo imaging assay.[5] Therefore, we[1] and others[6] have been exploring the development of the antisense ODN probes labeled with positron emitting fluorine-18 to image the biodistribution of ODNs and specific gene expression using PET. Fluorine-18 (96.9% β^+ emission), due to its close isosteric relationship with hydrogen,[7] offers a suitable alternative to mimic the biological behavior of the parent ODN. In this communication we report a rapid synthesis of 5′-fluoro-ODN that should be applicable for use with radiolabeled fluorine. The target antisense ODN is a 10-mer, d[C CGC CAG CTC], complementary to the 5′ translation start region of the her-2-neu proto-oncogene mRNA.[8] A high affinity is essential for the detection of an amplified oncogene mRNA that is present with a Bmax in the range of 1–1000 pM.[9] It has been reported that a deca-ribonucleotide binds to a single-stranded region of its complementary mRNA with affinity constants in the range of 0.01–0.1 pM.[10] In addition, a stretch of 10 nucleotide bases and high order structure requirements of hybridization should be enough to provide a high binding selectivity.[11] It is therefore conceivable that the antisense probe may detect even the lower level of target mRNA with a signal to noise ratio of ~10:1 (based on the ratio of Bmax to Kd at equilibrium). We have decided to use [^{18}F]fluoride and introduce it to the 5′-end of the above ODNs for the following reasons: (1) a compound with high specific activity (~10^3–10^4 Ci/mmol) can be attained with [^{18}F]fluoride,[12] which is neccesary for detecting relatively low levels of target mRNA; (2) the 5′-deoxy-5′-fluoro analogue of nucleoside has been shown to be stable under physiological condition;[13] (3) a fluorine-18 labeled nucleoside is introduced in the last step avoiding an extra radiation-exposure time and dilution of radioactivity; and (4) the half-life of ^{18}F is likely sufficient for kinetic determination of transport and specific binding as well as clearance of the unbound ODN.[14]

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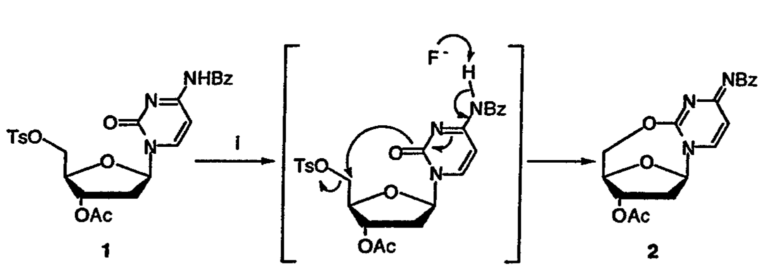
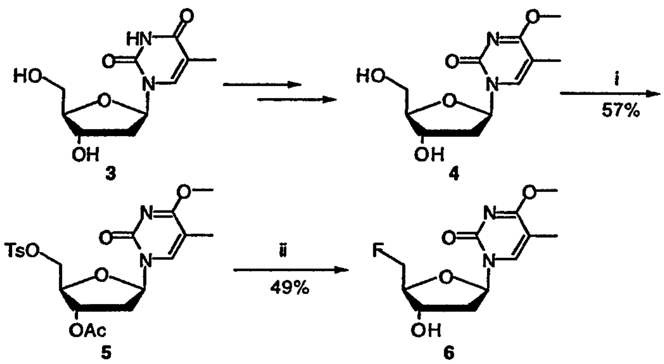
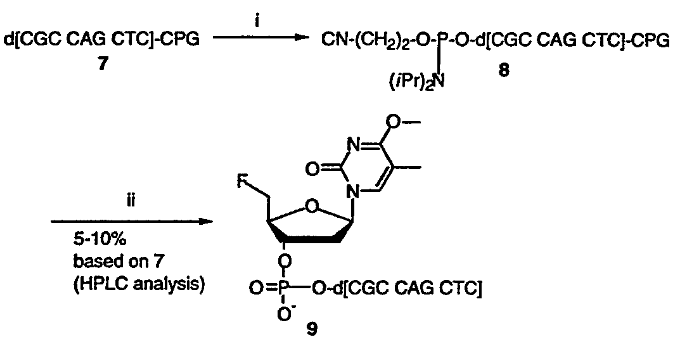


Scheme 1. *Reagents*: (i) KF/Kryptofix-[222], MeCN, 120 °C, 15.



Scheme 2. *Reagents*: (i) (1) TsCl, Py; (2) Ac_2O, Py; (ii) (1) KF/Kryptofix-[222], MeCN, 100 °C, 15 min; (2) conc NH_4OH, 100 °C, 15 min; (3) C_{18}-HPLC, MeOH:H_2O (40:60).

A number of elegant approaches to synthesize fluorinated nucleosides and nucleotides have been described.[15] In addition, a wide variety of reagents for fluorination are currently available.[16] Among these methods and reagents, only a few can be adapted to the specific constraints of ^{18}F-chemistry. These included the need to complete a series of reactions within 2–3 half-lives, after cyclotron production of the radionuclide, and the use of a large amount of radioactivity (~1 Ci) to compensate for radioactive decay and synthetic yields.[17] Our synthetic strategy is comprised of two key steps: synthesis of a 5′-deoxy-5′-fluoro-nucleoside followed by its incorporation into a CPG-bound ODN by the reverse-activation method introduced by Tan et al.[18]



Scheme 3. *Reagents*: (i) (iPr)$_2$NP(Cl)O(CH$_2$)$_2$CN, (iPr)$_2$EtN, 1-methylimidazole, Py, MeCN, rt, 1 h; (ii) (1) **6**, 1*H*-tetrazole, MeCN, rt, 30 min; (2) I_2, H_2O; (3) $MeNH_2$:NH_4OH (1:1), 50 °C, 10 min; (4) ion-exchange HPLC (POROS 20 HQ), buffer A: 23 mM Tris-HCl, 1 mM EDTA, pH 8.0 with H_2O:acetonitrile (90:10), buffer B: A containing 1.0 M NaCl, 10–60% B in 30 min.

First, the known 5′-*O*-tosyl derivative of cytidine[19] **1** was subjected to nucleophilic fluorination using KF and an azocrown ether, Kryptofix [222][20] (Scheme 1). The reaction, however, yielded only the 2,5′-anhydride **2** formed via nucleophilic attack by the 2-carbonyl oxygen initiated by proton abstraction from N^4 by fluoride.[21]

In order to avoid the intramolecular cyclization, we then chose the O^4-methylthymidine derivative **5**. O^4-Methylthymidine **4** acts as pseudo-cytidine by paring with guanosine.[22] According to a literature procedure,[23] thymidine **3** was converted to **4** in 37% yield (Scheme 2). Selective tosylation of **4** by the method of Reist et al.[24] followed by acetylation gave the precursor **5** in 57% yield. Fluorination was performed using two equivalents of KF and Kryptofix [222] in anhydrous MeCN at 100 °C in a sealed tube for 15 min.[25] The reaction mixture was subsequently treated with concentrated NH_4OH at 100 °C in a sealed tube for another 15 min. Purification by reverse-phase HPLC[26] afforded 5′-deoxy-5′-fluoro-O^4-methylthymidine **6** as a powder in 49% yield.[27] The structure was confirmed by ^{19}F NMR and HRMS.[26] Fluorination and purification were completed within 2 h.

Coupling of **6** to the CPG-bound 9-base ODN[28] **7** was carried out by the reverse-activation protocol[18] (Scheme 3). Phosphitylation of **7** was successful by treatment with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite and *N,N*-diisopropylethylamine in the presence of 1-methylimidazole and pyridine in anhydrous MeCN at room temperature for 1 h. The resulting phosphoramidite[29] **8** was then reacted with **6** in MeCN containing 1*H*-tetrazole at rt for 30 min. After oxidation with aqueous iodine, the product ODN was simultaneously deprotected and cleaved from the CPG following the standard $MeNH_2$-NH_4OH treatment at 50 °C for 10 min. The crude mixture was purified by ion-exchange HPLC (POROS 20 HQ) to yield the desired 5′-fluorinated ODN **9** in 5–10% yield based on **7** analyzed by HPLC.[30] The structure of **9** was confirmed by MALDI-TOF MS.[30] The total time required for coupling and purification was 2 h.

The present work demonstrates that the synthesis of 5′-fluorinated antisense ODN can be accomplished within 4 h, a neccesary condition for F-18 labeling. Since the fluorination of the nucleoside and the activation of CPG-bound ODN can be performed concurrently, the total reaction time could be reduced further. Synthesis of [^{18}F]fluorinated antisense ODN as well as its in vitro and in vivo applications will be reported elsewhere.

Acknowledgments. This work was supported in part by grants from the Department of Energy (DE-FC03-37ER60615), the UCLA-Jonsson Comprehensive Cancer Center, Dana Foundation, and the University of California Biotechnology Program. We would like to thank Dr. M. Namavari and Dr. R. Kodukulla for their helpful discussions and advice. We would also like to thank Ms. T. Sama for secretarial assistance.

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25 The condition has not been optimized.

26. Compound **6**: HPLC purification: column: Econosil C18 10U, 250 × 10 mm; gradient: methanol/H_2O (40/60); flow rate: 4.7 mL/min.; retention time: 10.8 min. ^{19}F NMR (Bruker AM360, $CDCl_3$, $CFCl_3$), δ -233.5. ^{1}H NMR spectra ($CDCl_3$, TMS) δ 1.95 (s, 3, OC*H*$_3$), 2.11 (br ddd, 1, $J_{H\text{-}2'a,H\text{-}2'b}$ = 13.5 Hz, $J_{H\text{-}2'a,H\text{-}3'}$ = 6.5 Hz, $J_{H\text{-}2'a,H\text{-}1'}$ = 4.0 Hz, H-2′a), 2.59 (ddd, 1, $J_{H\text{-}2'b,H\text{-}2'a}$ = 13.6 Hz, $J_{H\text{-}2'b,H\text{-}3'}$ = 5.6 Hz, $J_{H\text{-}2'b,H\text{-}1'}$ = 3.7 Hz, H-2′b), 3.57 (br s, 1, O*H*), 3.98 (s, 3, C*H*$_3$), 4.14 (dddd, 1, $J_{H\text{-}4',F\text{-}5'}$ = 33.0 Hz, $J_{H\text{-}4',H\text{-}3'}$ = 3.3 Hz, $J_{H\text{-}4',H\text{-}5'a}$ = 2.0 Hz, $J_{H\text{-}4',H\text{-}5'b}$ = 2.0 Hz, H-4′), 4.57 (m, 1, H-3′), 4.64 (ddd, 1, $J_{H\text{-}5'a,F\text{-}5'}$ = 48.5 Hz, $J_{H\text{-}5'a,H\text{-}5'b}$ = 10.7 Hz, $J_{H\text{-}5'a,H\text{-}4'}$ = 2.0 Hz, H-5′a), 4.72 (ddd, 1, $J_{H\text{-}5'b,F\text{-}5'}$ = 46.5 Hz, $J_{H\text{-}5'b,H\text{-}5'a}$ = 10.7 Hz, $J_{H\text{-}5'b,H\text{-}4'}$ = 2.0 Hz, H-5′b), 6.40 (dd, 1, $J_{H\text{-}1',H\text{-}2'a}$ = 4.0 Hz, $J_{H\text{-}1',H\text{-}2'b}$ = 3.7 Hz, H-1′), 7.59 (s, 1, H-6). HRMS (electrospray) $[M+H]^+$, obsd: 259.1092, calcd: 259.1094.

27 The yield was calculated basing on 4-*O*-methyl-3′-*O*-acetyl-5′-*O*-tosyl thymidine **5**.

28. The CPG-bound ODN **7** was prepared on a Beckman 1000M DNA synthesizer, following standard phosphoramidite chemistry.
29. The quality of the CPG-bound phosphoramidite **8** can be evaluated as described in ref 11.
30. 5′-Fluorinated ODN **9**. HPLC purification: column: POROS 20 HQ, 100 × 4.6 mm; eluent A: H_2O, 25 mM Tris-HCl, 1 mM EDTA, pH 8.0 with H_2O/acetonitrile (90/10); eluent B: A plus 1 M NaCl; gradient: 20–50% B in 15 min; flow rate 4 mL/min; retention time: 7.3 min. MS (MALDI–TOF)$[M + H]^+$, obd: 2979, calcd: 2980.

uptake was similar for the two radiolabels, and in most normal tissues the ^{86}Y and ^{111}In concentrations differed by less than 10%. In bone, however, the ^{86}Y uptake exceeded that of ^{111}In by 19±6%. **Conclusions:** The quantitative information offered by PET, combined with the presumably identical biodistribution of an ^{86}Y and an ^{90}Y radiolabel, should enable more accurate absorbed dose estimates in ^{90}Y radioimmunotherapy.

No. 329

PREPARATION AND *IN VIVO* STABILITY EVALUATION OF LINKERS FOR ^{211}AT LABELING OF HUMANIZED ANTI-TAC(ANTI IL-2Rα). K. Garmestani*, A. T. Yordanov, K. E. Phillips, M. P. Beitzel, U. P. Schwarz, M. S. Rickford, O. A. Gansow, P. S. Plascjak, W. C. Eckelman, M. W. Brechbiel, T. A. Waldman, National Institutes of Health, Bethesda, MD. (100480)

Objectives: Radionuclide-labeled Mabs directed against tumor-associated antigens have been investigated as immunotherapeutic agents in human cancer. The α-emitter ^{211}At ($t_{1/2}$ = 7.21 h) is a particularly promising candidate for radioimmunotherapeutic applications. A pivotal issue to consider in designing an optimal radioimmunotherapeutic agent is the choice of linker to couple the radionuclide to the MAb. **Methods:** The linkers *N*-hydroxysuccinimidyl 4-[^{211}At]astatobenzoate(**1**), *N*-hydroxysuccinimidyl 3-[^{211}At]astato-4-methylbenzoate(**2**), *N*-hydroxysuccinimidyl 4-[^{211}At]astato-3-methylbenzoate(**3**) and *N*-hydroxysuccinimidyl *N*-(4-[^{211}At]astatophenetyl)succinamate(**4**) were prepared and employed for ^{211}At-labeling of the antibody. The anti-Tac (anti IL-2Rα) antibody that reacts with select leukemia cells but not resting normal cells was utilized for this study. The plasma survival of these compounds in normal mice was studied vs ^{125}I labeled humanized anti-Tac. **Results:** The comparison of the blood clearance curves of the ^{211}At and ^{125}I-labeled anti-Tac and free ^{211}At indicating the stability of compounds **1-4** was in the following order $^{125}I \cong \mathbf{4 > 3 > 2 > 1}$. **Conclusion:** This study showed that linker **4** is the superior compound prepared to date for ^{211}At-labeling of humanized anti-Tac and its plasma survival appeared to be essentially equivalent to that of directly labeled ^{125}I-antibody. These results also suggest that humanized anti-Tac can be successfully labeled with ^{211}At using linker **4** and should be further evaluated for therapeutic applications.

Radiopharmaceutical Chemistry Track New Chemistry: Oncology - Hypoxia, Nucleosides

4:00 PM-5:30 PM Session 48 Room: 403 B

Moderator: Chyng-Yann Shiue, PhD
Co-Moderator: Janet F. Eary, MD

No. 330

NON ENZYMATIC REDUCTION AS A POSSIBLE RETENTION MECHANISM OF TC-99M-HL91 IN HYPOXIC TISSUES. Y. Fujibayashi*, M. Ohno, A. Waki, K. S. Horiuchi, Y. Yonekura, Fukui Medical University, Fukui, Japan; Kyoto University, Kyoto, Japan. (210)

A novel hypoxia imaging agent, Tc-99m-4,9-diaza-3,3,10,10,-tetramethyldodecan-2,11-dione dioxime (Tc-99m-HL91), shows hypoxia-selective accumulation in myocardium as well as tumors, but its retention mechanism has not been clarified. In our previous work, it was found that Cu-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) showed enzymatic and NADH-dependent reduction in hypoxic non-tumor tissues. Thus, in the present work, metabolic analysis was performed to clarify the reductive retention mehcanism of Tc-99m-HL91 in in-vitro system. Methods: Metabolism of Tc-99m-HL91 and Cu-ATSM was comparatively evaluated using reversed-phrase HPLC system. Each sample was incubated with biological reductants, glutathione reduced form or NADH, then analyzed. For controls, oxidized forms of the reductants were used. To evaluate the possible contribution of enzymatic systems, subcellular fractions obtained from Ehrlich ascites tumor cells were added to the incubation medium. The effect of NADH on the enzymatic reduction of each samples was also studied. Results: The reduction of Cu-ATSM required microsomal enzymes and was NADH/NADPH dependent in tumor cells. Without enzymes, no reduction could be found. On the other hand, Tc-99m-HL91showed chemical reduction when only NADH or glutathione reduced form was added to the incubation medium. This reduction was dose-dependent, but there seemed to be threshold levels of reductant concentration. More interestingly, enzyme system inhibited the reductive metabolism of Tc-99m-HL91 but electron transport inhibitors recalled the reduction of Tc-99m-HL91 in the medium containing the microsomal enzymes. Conclusion: Cu-ATSM could be considered as a marker of reversible hypoxia, bacause it required biological reductants as well as intact enzyme system(s). On the other hand, Tc-99m-HL91 only required abnormally high concentration of biological reductants, indicating it as a hypoxia imaging agent with wider spectrum, rather than Cu-ATSM. This finding will bring us a new sight of hypoxia diagnosis using Tc-99m-HL91 as well as Cu-ATSM in clinical level.

No. 331

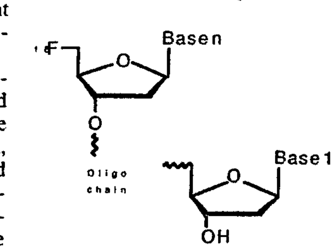
TARGETTING HYPOXIA IN TUMOURS USING 2-NITROIMIDAZOLES WITH PEPTIDIC CHELATORS FOR TECHNETIUM-99M: EFFECT OF LIPOPHILICITY. X. Zhang, Z. F. Su, J. R. Ballinger*, A. M. Rauth, A. Pollak, J. R. Thornback, Ontario Cancer Institute/University of Toronto, Toronto, ON, Canada; Resolution Pharmaceuticals, Mississauga, ON, Canada. (100536)

Objectives: Hypoxia in tumours is an important prognostic factor for response. Radiolabelled 2-nitroimidazoles (2-NI) have been used for imaging hypoxia and partition coefficient (*P*) appears to play a crucial role in suitability for imaging. We developed a series of eleven 2-NI containing a peptidic chelator for ^{99m}Tc with divergent *P* and evaluated them in an *in vitro* system. **Methods:** Two classes of N_3S chelators were used: dialkyl-Gly-Ser-Cys-linker-2-NI and dialkyl-Gly-Lys(2-NI)-Cys. Prepared by automated solid-phase peptide synthesis, the chelators were labelled by transchelation from ^{99m}Tc-gluconate at temperatures between 20 and 100°C. The reaction mixtures were analysed by HPLC. The accumulation of each complex in suspension cultures of Chinese hamster ovary cells incubated under aerobic or extremely hypoxic conditions was determined. **Results:** Radiochemical yields ranged from 5% to 80% for the 11 compounds. HPLC showed that some compounds formed two complexes with ^{99m}Tc, possibly syn- and anti-conformations with respect to the Tc=O bond. In general, the Gly-Ser-Cys chelator labelled more readily than the Gly-Lys-Cys chelator. The *P* values varied from 0.001 to 5, and were generally in accordance with predictions based on structure. There were also differences in *P* as a function of pH; the free acids had a lower *P* at pH 7.4 than at pH 2.0 due to ionisation, whereas the amides did not show this effect. Accumulation levels in cells were related to *P* but varied over a narrower range. Six of the 11 compounds showed selective localisation in hypoxic cells, with 1.8- to 3.6-fold higher accumulation in hypoxic *vs* aerobic cells. **Conclusions:** The peptidic class of 2-NI, with flexible and convenient solid-phase synthesis, deserves further study as agents for imaging hypoxia in tumours.

No. 332

SYNTHESIS OF A FLUORINE-18 LABELED ANTISENSE OLIGODEOXYNUCLEOTIDE AS A PROBE FOR IMAGING GENE EXPRESSION. D. Pan*, T. Toyokuni, J. R. Barrio, N. Satyamurthy, M. E. Phelps, S. S. Gambhir, University of California at Los Angeles School of Medicine, Los Angeles, CA. (500498)

We are developing methods to image gene expression *in vivo* by positron emission tomography (PET). Antisense oligodeoxynucletoides (ODN) and their derivatives complementary towards a small region of mRNA are being studied for targeting the mRNA of various amplified oncogenes. Here, we describe the synthesis of a PET ODN probe in which 5′-OH group of the ODN is replaced by [F-18]fluorine. The synthesis involves radiofluorination of a modified nucleoside followed by its coupling to a fully protected CPG-bound ODN. The key precursor, 5′-*O*-tosyl-3-*O*-di (*p*-methoxyphenyl) phenylmethyl-4-*O*-methyl-thymidine, was prepared in six steps from thymidine in a 22% overall yield. Nucleophilic fluorination of the precursor with [F-18]fluoride ion in the presence of K+/Kryptofix and subsequent deprotection gave 5′-deoxy-5′-[F-18]fluoro-4-*O*-methyl-thymidine. Coupling to the 5′-phosphoramidite-activated CPG-bound ODN, simultaneous cleavage from the CPG and de-protection, and HPLC purification furnished the target 5′-deoxy-5′-[F-18]fluoro-ODN probe. The HPLC spectrum was identical to that of the



F-19 counterpart. The replacement of 5′-OH by a fluorine atom did not cause any significant changes in hybridization affinity to complementary RNA sequence as determined by measurements of Tm. Biodistribution studies with various fluorine-18 labeled ODN derivatives in mice imaged in a microPET are currently underway.

No. 333

AN IMPROVED SYNTHESIS OF 9-[(3-[F-18]FLUORO-1-HYDROXY-2-PROPOXY)METHYL]GUANINE ([F-18]FHPG). C.-Y. Shiue*, G. G. Shiue, R. Hustinx, A. A. Alavi, S. L. Eck, University of Pennsylvania, Philadelphia, PA. (500488)

Gene transfer, especially herpes simplex virus thymidine kinase gene transfer has shown significant potential in treating several common cancers. The principal obstacle to successful gene therapy has been the development of genetic vectors capable of achieving efficient gene transfer and the methods of assessing their transfers *in vivo* non-invasively. [F-18]FHPG(1) has been synthesized and suggested as a potential agent for monitoring the efficiency of gene therapy. The purpose of this study was to improve and simplify the synthesis of [F-18]FHPG. **Methods:** [F-18]FHPG was synthesized by nucleophilic substitution of N2-(p-Anisyldiphenylmethyl)-9-[[1-(p-anisyldiphenylmethoxy)-3-toluenesulfonyloxy-2-propoxy]methyl]guanine with [F-18]fluoride at different temperatures. The resulting intermediate was deprotected in 1N HCl at different temperatures and the product was isolated with either HPLC (Alltech, C18, 10x250 mm; CH3CN/H2O; 5/95; 2 mL/min) or silica Sep-Pak (The by-product was washed out first with CH2Cl2/MeOH, 9/1 and the product **1** was isolated with CH3CN/H2O, 8/2). For stability studies, **1** was dissolved in 1N HCl and heated at 90°C and 120°C, respectively, for different time intervals and monitored with TLC. *In vitro* activity of **1** synthesized and purified by HPLC and TLC was evaluated with 9L (gliomas) cells. **Results:** The yield of **1** decreases as the reaction temperature increases. At 120°C and 90°C, and the product was purified with HPLC, the yield of **1** was 2 and 5-10%, respectively. The synthesis time was 90 min. from EOB. The yield of **1** increased to 10-15% when the reaction temperature was 90°C and the product was purified by silica Sep-Pak. The synthesis time was 60 min from EOB. [F-18]FHPG was unstable in 1N HCl at high temperature. At 120°C, 50% of **1** was decomposed in 10 min while 90% of **1** remained intact at 90°C. [F-18]FHPG purified either by HPLC or silica Sep-Pak has the same *in vitro* activity. **Conclusion:** The yield of [F-18]FHPG can be improved by carrying out the reaction at lower temperature (90°C instead of 120°C) and purified with silica Sep-Pak. The same procedure probably can be applied to prepare similar radiotracers (eg. penciclovir).

No. 334

SYNTHESIS OF F-18 2-FLUORO-5-METHYL-1-β-D-ARABINOFURANOSYLURACIL ([F-18]FMAU). P. S. Conti*, M. M. Alauddin, J. D. Fissekis, K. A. Watanabe, University of Southern California, Los Angeles, CA; Memorial Sloan-Kettering Cancer Center, New York, NY. (500282)

Objectives: 2′-Fluoro-5-(C-11)methyl-1-β-D-arabinofuranosyluracil (C-11 FMAU) is a potential marker for cell proliferation by positron emission tomography (PET). The presence of the fluorine atom at the 2′-position prevents catabolism *in vivo*. The short half-life of C-11 and the air-sensitive organometallic synthesis, limits the production and use of the C-11 compound. Fluorine-18 labeled FMAU may potentially be more advantageous in certain applications. The direct, stereospecific (*arabino*) introduction of fluorine at the 2′-position of the furanosyl moiety in a uracil nucleoside has not been possible. Here we are exploring the incorporation of F-18 fluorine at the C-2 (*arabino*) position of the sugar followed by coupling with the pyrimidine. **Methods:** 2-Fluoro-1,3,5-tri-O-benzoyl-α-D-ribofuranose was coupled with thymine silyl ether in MeCN using $SnCl_4$ by heating at 70°C for 40 minutes. The coupled product was characterized by NMR spectroscopy, then hydrolyzed by NaOM in MeOH. FMAU was purified by HPLC and characterized by NMR spectroscopy. Radiosyntheses were performed with F-18 2-fluoro-1,3,5-tri-O-benzoyl-α-D-ribofuranose which was prepared following a method developed in our laboratory. The F-18 fluoro-sugar was purified by HPLC, dried and coupled with thymine silyl ether. The crude coupled product was extracted with CH_2Cl_2, evaporated and heated with NaOMe in MeOH for 7 min to hydrolyze the protecting groups in the sugar moiety. **Results:** The coupling reaction produced a mixture of α- and β-isomers which could be separated by HPLC. Radiolabed FMAU was isolated by HPLC purification using 7.5% MeCN in water. The product was co-eluted with an authentic sample of unlabeled FMAU. In preliminary runs (n=3) the decay corrected radiochemical yield was low (2-5%) although radiochemical purity was >99%. Synthesis time was 3.75h from the end of bombardment. **Conclusion:** F-18 FMAU has been successfully prepared although optimization of reaction conditions is required in order to improve yield.

No. 335

OPTIMIZING LABELING SUBSTRATE STRUCTURE FOR 3′-DEOXY-3′-[F-18]FLUOROTHYMIDINE: [F-18]FLT. J. R. Grierson*, A. F. Shields, University of Washington, Seattle, WA; Wayne State University, Detroit, MI. (500381)

Objectives: We recently demonstrated that FLT can be used with PET to provide images of proliferation in vivo. We have established a working synthesis for [F-18]FLT. however, we desired a more efficient process suitable for multi-dosing. **Methods:** Our original labeling substrate for [F-18]FLT synthesis was compound (1): 1-(2-deoxy-3-O-methanesulfonyl-5-O-(4,4′-dimethoxytrityl)-β-D-threo-pentofuranosyl)-3-(2,4-dimethoxybenzyl)thymine. We compared the labeling of a variety of newer substrates against (1) to discover an optimum structure (see Table). Trial labeling experiments were done with the same batch of pre-solubilized fluoride (substrate/KRY/carbonate 1.6-2:2:1) in MeCN, and portions were used (some sets used different batches but always included Cpd (1) for comparison, no yields included unless Cpd 1 gave >20 percent). Production level experiments = yields in parentheses (substrate/KRY/carbonate 1.3:2:1) were done with whole batches of dried, complexed fluoride. **Results** (Table) **and Conclusions:** The N-alkylated substrate performed better than the N-acylated case (1 vs. 2). Of all the leaving groups used the 3-NO2-PhSO2-ester was superior, because the 4-NO2-PhSO2-ester was not displaced from the sugar, rather, the 4-NO2-on the leaving group itself was displaced from the aromatic ring (i.e. nucleophilic aromatic substitution). The triflate compound (5) yield was low, due to its poor stability during synthesis.

Labeling substrate structure and r.c. yields (decay corr.)

Compound	N-group	5-O-group	3-O-ester	Average yield
1	2,4-Di-MeOBn	Di-MeO-trityl	mesylate (Mes)	23.5 (17)
2	Alloxycarbonyl	Di-MeO-trityl	mesylate	17 (10)
3	2,4-Di-MeOBn	Di-MeO-trityl	3-NO2-PhSO2-	42
4	p-MeOPh	p-MeOPh	4-NO2-PhSO2-	(60)-not FLT
5	Alloxycarbonyl	Di-MeO-trityl	triflate	21

No. 336

A METABOLIC STUDIES OF 18F- ALPHA-METHYL TYROSINE: FRACTIONATION OF ITS INCORPORATION INTO BRAIN AND TUMOR IN MICE BEARING LCI-180. K. Tomiyoshi*, T. Inoue, K. Endo, Gunma University, Maebashi, Japan. (212)

Objectives: 18F- alpha-methyl tyrosine(18FAmT) has been clinically used and proved to be a very promising agent as determined from our PET studies. However little information on metabolism of 18FAmT is known. We investigated the metabolism in tumor and brain of mice bearing LCI-180 colorectal carcinoma. Methods : Homogenized tissues of brain and tumor in postinjection of 18FAmT at 5, 30 and 60 minutes were analyzed by fractionation method into acid soluble fraction(ASF) and acid precipitable fraction(APS). APS was further investigated to assess the incorporation of 18FAmT into each fraction by HPLC and TLC. Results : 18FAmT was stable up to 6 hours in Saline and plasma in vivo study. Incorporation into four fractions of brain and tumor at 60 minute post injection were 20% and 12%. Among them, 10% of the activity were incorporated to lipid in brain and 5% in tumor. There was 5%, 2%, 2% in RNA, DNA and protein. Conclusion : The uptake of 18FAmT in tissue was rapid and accomplished before 30 minutes and then slowly diffused in blood. These results implied that 18FAmT was little metabolized to protein and trapped as intact 18FAmT in cell up to 60 minutes. 18FAmT is promising tracer for imaging and quantification of transport rate using two compartment models.

APPLICATION OF SILICON–FLUORIDE CHEMISTRY TO FLUORINE-18 LABELING AGENTS FOR BIOMOLECULES: A PRELIMINARY NOTE

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Key Words: fluorine-18, labeling agent, imaging, PET, silicon.

Positron-labeled biomolecules such as nucleotides, peptide/carbohydrate ligands and antibody fragments are of great interest due to their ability to quantitatively detect and characterize a variety of disease processes in living humans using positron emission tomography (PET). Among a number of positron-emitting nuclides, fluorine-18 (^{18}F) is most suitable for labeling such biomolecules. ^{18}F has the longest half-life (110 min) of the routinely available positron emitters and decays nearly 100% by positron emission with weak positron energy. Several ^{18}F-labeling agents have been developed and used to label proteins [1,2,3] and oligonucleotides [4]. All the reported procedures involve pre-activation of cyclotron-produced aqueous [^{18}F]fluoride (i.e., aqueous H[^{18}F]) by evaporation from an added base such as K_2CO_3/Kryptofix-222 and n-$Bu_4N^+OH^-$. This evaporation step often occupies a substantial fraction of the overall reaction time. Furthermore, due to a large excess of added base, subsequent nucleophilic [^{18}F]fluorination must be carried out under strong basic conditions. Such conditions are not suitable for compounds containing an amine- or a thiol-reactive group (e.g., *N*-hydroxysuccinimide esters and maleimides, respectively) as well as biomolecules. Although two amine-reactive ^{18}F-labeling agents have been prepared by direct [^{18}F]fluorination of appropriate active esters, the methods suffer from either low specific activity [5] or the need of extensive HPLC purification [6]. Reductive alkylation of an amino group might be an alternative method for labeling proteins, since [^{18}F]fluorobenzaldehyde can be prepared by nucleophilic aromatic [^{18}F]fluorination of a benzaldehyde derivative (e.g., a trimethylammonium triflate salt) with pre-activated [^{18}F]fluoride [7]. We have initiated a program to develop novel ^{18}F-labeling agents that can be [^{18}F]fluorinated with cyclotron-produced aqueous H[^{18}F] without the pre-activation step. It is reported that aqueous HF is a weak acid (Ka 6.46 x 10^{-4} mole/L) [8]. Therefore, direct use of aqueous H[^{18}F] will not only save considerable reaction time but may also provide a mild fluorination condition compatible with the presence of the amino- and thiol-reactive groups and with biomolecules. In view of the ease of the Si–F bond formation in an aqueous environment as demonstrated by Whitmore et al. [9] and Gatley et al. [10], we are currently exploring silicon–fluoride chemistry to achieve our goal. In this presentation a prototype of a silicon-based ^{18}F-labeling agent as well as its application to the development of a thiol-reactive ^{18}F-labeling agent is described.

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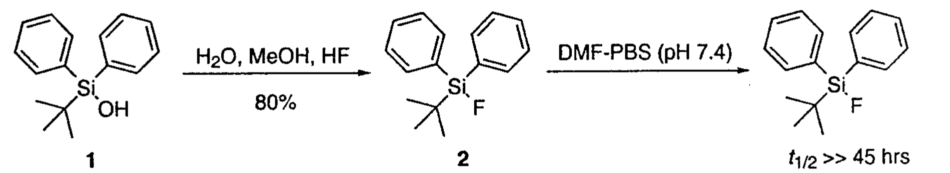
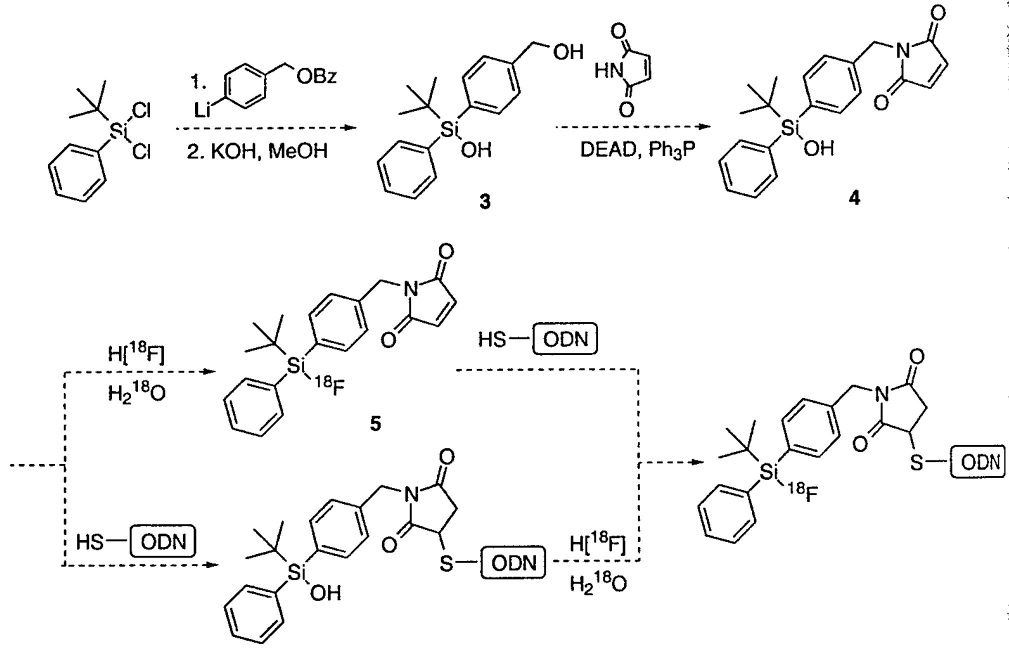
In order for the silicon-based ^{18}F-labeling agent to be effective as a PET probe, the Si–F bond must be resilient towards hydrolysis at physiological pH (i.e., blood plasma pH 7.35–7.45). It is well documented that the hydrolytic stabillity of the silicon–halogen bond is determined by the nature of the alkyl substituents on silicon [11,12]. Based on these considerations, it was determined that both phenyl and *tert*-butyl groups were needed to afford the stability. We synthesized a model fluorosilane using non-radioactive fluoride ($^{19}F^-$) in order to test our hypothesis (Scheme I). The silanol **1**, prepared from *tert*-butyldiphenylchlorosilane and KOH in aqueous methanol, was reacted with a sub-stoichiometric amount of HF in aqueous methanol at 0°C for 10 minutes to yield the fluorosilane **2** in 80% yield [13,14]. The stability of the Si–F bond in **2** was then examined with the aid of ^1H, ^{13}C and ^{29}Si NMR spectroscopy after treatment with DMF containing an excess amount of PBS (pH 7.4) at room temperature. No observable hydrolysis of the Si–F bond was seen after 3 h. Even after 45 h of exposure to PBS, over 95% of the fluorosilane remained in tact.

Scheme I



Scheme II



Encouraged by the results performed on the model system, we are developing a thiol-reactive ^{18}F-labeling agent for a thiol-modified oligodeoxynucleotide (ODN) (Scheme II). Our synthetic plan involves derivatization of one of the aryl groups in such a way that a thiol-reactive maleimide group is introduced to the silanol **3**. Two ^{18}F-labeling approaches will be considered. The first approach is a two-step [^{18}F]fluorination in which the thiol-reactive labeling agent **4** is first [^{18}F]fluorinated with H[^{18}F] and the resulting **5** is conjugated with a ODN. The second approach is a direct [^{18}F]fluorination of the preformed conjugate of **4** and the ODN. The ^{18}F-labeled antisense ODN may become a useful PET probe in biology and medicine to image specific gene expression in living subjects [4,15]. Simple ^{18}F-labeling methods are therefore urgently needed.

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No. 1080

IN VIVO LABELING OF ENDOTHELIN RECEPTORS WITH [^{11}C]L-753, 037. S. Aleksic*, Z. Szabo, U. Scheffel, H. T. Ravert, O. Vaterlein, L. Kerenyi, R. E. Gibson, C. Ryan, T. Hamill, H. D. Burns, and R. F. Dannals, The Johns Hopkins Medical Institutions, Baltimore, MD; Department of Radiopharmacology, Merck Research Laboratories, West Point, PA. (500367)

Objectives: Endothelin (ET), a potent vasoconstrictive peptide, acts by binding to two major receptor subtypes, ET-A and ET-B. These receptors have not yet been imaged in vivo by PET. This study was undertaken to determine if [^{11}C]L-753, 037, [(+)-(5S, 6R, 7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3, 4-methylenedioxyphenyl) cyclopenteno [1, 2-b]pyridine-6-carboxylate], a new mixed ET-A/ET-B receptor antagonist, could be used to label endothelin receptors in vivo. **Methods:** [^{11}C]-L-753, 037 was synthesized by [^{11}C]-methylation of a phenolic precursor, L-843, 974. Its in vivo kinetics, biodistribution, and binding characteristics were evaluated in mice. The specificity of receptor binding was assessed using the selective ET-A antagonist, L-753, 164. **Results:** Kinetic studies in mice showed the highest tracer uptake at 5 min post-injection (p.i.) in the liver (25.0 % injected dose; ID/g), followed by kidneys (18.7 %ID/g), lungs (15.2 %ID/g) and heart (5.6 %ID/g). Initial uptake in liver, kidneys and lungs was followed by a rapid wash-out during the next 10 min and then by a very slow clearance up to 2 hours p.i. By contrast, the activity in the heart remained almost unchanged over 2 hours. Administration of 1 mg/kg of both L-753, 164 (ET-A selective antagonist) and L-753, 037 (mixed ET-A/ET-B antagonist), resulted in significant inhibition of [^{11}C]L-753, 037 binding in mouse heart, lungs, kidneys and adrenal glands. Inhibition by L-753, 164 in the heart was dose-dependant (16, 72 and 96% at 0.1, 1.0, and 10 mg/kg, respectively). In the dog, a dynamic PET study of the heart showed high tracer accumulation at 55-95 minutes p.i. Pre-injection of L-753, 164 (1.0 mg/kg) 30 min before [^{11}C]L-753, 037 administration, led to a 58% reduction in tracer binding at 85 min. **Conclusion:** The results suggest that [^{11}C]L-753, 037 binds to endothelin receptors in vivo and is a promising candidate for investigation of these receptors by PET.

No. 1081

USE OF 5′-DEOXY-5′-[^{18}F]FLUORO-4-O-METHYLTHYMIDINE IN THE SYNTHESIS OF ^{18}F-LABELED ANTISENSE OLIGODEOXYNUCLEOTIDE PROPBES FOR IMAGING GENE EXPRESSION WITH PET. J. C. Walsh*, D. Pan, N. Satyamurthy, J. R. Barrio, M. E. Phelps, T. Toyokuni, and S. S. Gambhir, University of California at Los Angeles School of Medicine, Los Angeles, CA. (500601)

We have been involved in the development of ^{18}F-labeled oligodeoxynucleotides (ODNs) for the use as PET antisense probes to image mRNA expression in living subjects. Our approach is to synthesize 5′-deoxy-5′-[^{18}F]fluorinated nucleoside which is then coupled to a pre-assembled ODN on a solid-support using the reverse-activation protocol. We have previously reported preliminary results on the synthesis of an ^{18}F-labeled ODN (16-mer) using this approach. We now present detailed examination of the synthesis identifying some problems associated with our approach. Synthesis of 5′-deoxy-5′-[^{18}F]fluoro-4-O-methylthymidine occurs efficiently yielding ~25% radiochemical yield (decay corrected) with >99% radiochemical purity. However, subsequent coupling of 1 to a pre-assembled ODN (15 mer), followed by deprotection and purification of the ^{18}F-ODN (16 mer), is found problematic. First, the coupling is inefficient due to competing hydrolysis of the phosphoramidite by trace quantities of water. The radiochemical yield is generally in the range of 0.01–0.1% (decay corrected). Second, deprotection of the ^{18}F-ODN using conventional AMA reagent (NH4OH–MeNH2) at 65 °C for 7–10 min is accompanied by partial de[^{18}F]fluorination. It seems that amine bases attack the O4-methylthymine portion to form the 5-methylcytosine derivatives, which then cyclize to the 2, 5′-anhydride releasing [^{18}F]fluoride. On the basis of HPLC analysis, 10-min exposure of the protected ^{18}F-ODN to the AMA reagent gives rise to radioactivity corresponding to [^{18}F]fluoride, partially deprotected ^{18}F-ODN and the desired ^{18}F-ODN in a ratio of 1.4 : 1.8 : 1.0. The ratio can be improved to 1.0 : 6.6 : 2.3 by 7-min exposure. Third, it is difficult to separate the ^{18}F-ODN from the unreacted 15-mer resulting in the low specific activity of <500 Ci/mmol (decay corrected). We are currently addressing these problems and the progress will be presented.

No. 1082

NEW N_3S LIGANDS FOR ^{99m}Tc RENAL IMAGING. M. Lipowska*, L. Hansen, L. G. Marzilli, and A. Taylor, Emory University, Atlanta, GA. (100232)

Objectives: To develop a ^{99m}Tc renal imaging agent with the clearance equivalent to ^{131}I OIH by using new N_3S ligands. **Methods:** The tetradentate chelates N-(2-(pyridylamido)ethyl)-L-(or D-)cysteine (PAEC) were synthesized in high yields by condensation of N-(2-aminoethyl)-L-(or D-)cysteine with succinimidylpicolinate and characterized. PAEC ^{99m}Tc labeling by the Glucoscan kit method (pH 8-11) afforded *syn* and *anti* isomers. Biodistribution, renal clearance (Cl) and renal extraction fraction (EF) studies of the *syn*-TcO(L- and D-PAEC) were performed in rats (n = 6) using OIH as an internal control. The Re derivative of PAEC was prepared by ligand exchange reaction with $ReO_2I(PPh_3)_2$. **Results:** Four TcO(PAEC) stereoisomers are possible because the new ligand is chiral and the complex forms *syn* and *anti* isomers. Two radiochemical products (3:1 ratio) were obtained at the optimal labeling conditions (pH 8). This ratio can be increased by using higher pH, but the combined yield (*syn* + *anti*) is lower. The *anti* isomers were unstable (~ 80% decomposed at 3 h) and thus were not included in the animal studies. *syn*-TcO(L-PAEC) was efficiently extracted by the kidney (65±4% of the dose was found in the kidneys and bladder 30 minutes post injection; the clearance was 75±6% and the extraction fraction was 91±13% that of OIH). In comparison, *syn*-TcO(D-PAEC) had a clearance and EF only 33±3% and 52±13% of OIH, respectively. ReO(L-PAEC) obtained in 23% yield was a mixture of *syn* and *anti* isomers (5:1 ratio) as revealed by ^{1}H NMR and HPLC analysis. **Conclusion:** PAEC ligands form ^{99m}Tc and Re complexes as a mixture of diastereomers (*syn* and *anti*) at pH 8. The *syn*-TcO(L-PAEC) isomer shows good renal clearance characteristics but the need for HPLC purification limits its use as a clinical renal imaging agent.

No. 1083

INDIUM AND COPPER FORCEFIELDS SUITABLE FOR THE MOLECULAR MODELLING OF LABELLED BIFUNCTIONAL CHELATE PEPTIDE CONJUGATES. D. E. Reichert*, P. O. Norrby, and M. J. Welch, Washington University School of Medicine, St. Louis, MO; Royal Danish School of Pharmacy, Copenhagen, Denmark. (100184)

Molecular mechanics parameters for In(III) and Cu(II) have been developed for the AMBER force field, as implemented within the commercial package MacroModel, based on crystallographic and *ab initio* data. These parameters were then utilized in a study of the conformational preferences of several small peptides and their metal bound bifunctional chelate (BFC) conjugates. Octreotide is a synthetic octapeptide analog of somatostatin, which when conjugated to various BFC's such as DTPA, DOTA, and TETA and radiolabelled with various radionuclides such as ^{111}In, ^{90}Y, and ^{64}Cu has found use in imaging and radiotherapy of somatostatin receptor positive tumors. This cyclic peptide and several analogs of octreotide, such as octreotate with the terminal threonol replaced by threonine, and Tyr^3-octreotide with the Phe^3 replaced by tyrosine were modelled with this force field. These studies were performed using the GB/SA aqueous solvation model, in order to examine the conformational preferences of the parent peptides in a more realistic environment than the usual vacuum. The studies were then repeated with BFC conjugates (DTPA and DOTA) of these peptides labelled with both In(III) and Cu(II). The results from these studies indicate that the choice of radiometal and bifunctional chelate can have significant effects on the conformational preferences of the peptide and therefore on binding to the targeted receptor.

No. 1097

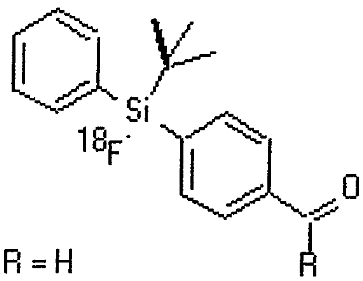
DIFFERENT CHELATORS (HYNIC, DTPA, MAG3) INFLUENCE THE BEHAVIOR OF ^{99m}Tc IN CELL CULTURE WHEN USED TO RADIOLABEL ANTISENSE DNA. Y. M. Zhang*, N. Liu, and D. J. Hnatowich, University of Massachusetts Medical School, Worcester, MA. (500221)

We have shown recently that cell accumulation in culture of antisense DNA is strongly influenced by the presence of a ^{99m}Tc-MAG3 group for radiolabeling. **Objectives:** In this investigation, we have compared the in vitro behavior of ^{99m}Tc when radiolabeled to one antisense uniform phosphorothioate DNA (i.e. 5′-GCGTGCCTCCTCACTGGC) by three different methods. **Methods:** An 18-mer antisense DNA against the RIa subunit of PKA was obtained with a primary amine on the 5′ end via a 6-member alkyl linker. The amine was conjugated with the NHS esters of HYNIC and MAG3 and by the cyclic anhydride of DTPA. **Results:** By surface plasmon resonance, the association rate constants for hybridization to the uniformly phosphorothiolated sense DNA was unchanged by the conjugation from that of unconjugated antisense DNA in the case of HYNIC and MAG3, but was significantly reduced by conjugation with DTPA, possibly because of anhydride attack on the nitrogenous bases. Labeling efficiencies and specific activities for ^{99m}Tc were highest for HYNIC (tricine) and MAG3 compared to DTPA while stability to cysteine transchelation was in the order HYNIC>DTPA>MAG3. Incubation of labeled DNA in 37°C serum and cellular media showed protein binding by size exclusion HPLC in the order HYNIC>MAG3>DTPA with the phosphorothioate backbone presumably contributing. In each case, radiolabeled and intact DNA was still detectable after 24 hrs. To test cellular uptake, ACHN tumor cells were used after RT-PCR showed that the RIa mRNA is expressed in this cell line. The order of cellular accumulation of ^{99m}Tc was DTPA>hynic>MAG3 with the differences becoming more significant with time between about 4-24 hrs. The rate of ^{99m}Tc egress from cells was found to be MAG3>HYNIC>DTPA which partially explains the order of cellular accumulation. **Conclusion:** Although these results were obtained for one antisense DNA in one cell type, we conclude that the success of antisense imaging may depend, in part, on the method of radiolabeling. This investigation was conducted with financial support from the US Department of Energy.

No. 1098

APPLICATION OF SILICON-FLUORIDE CHEMISTRY FOR THE DEVELOPMENT OF AMINE-REACTIVE ^{18}F-LABELING AGENTS FOR BIOMOLECULES. J. C. Walsh*, L. M. Fleming, N. Satyamurthy, J. R. Barrio, M. E. Phelps, S. S. Gambhir, and T. Toyokuni, University of California at Los Angeles School of Medicine, Los Angeles, CA; University of California at Los Angeles, Los Angeles, CA. (500296)

Target-specific imaging agents are becoming increasingly important for the non-invasive, in vivo diagnostics of various diseases. Targeting molecules include receptor-specific peptide ligands, oligonucleotides and antibody fragments. Although PET provides better image quality and resolution than SPECT, most target-specific imaging agents under development are those labeled with ^{99m}Tc for SPECT imaging. This is partially due to the ease of ^{99m}Tc labeling that involves a mixing of a targeting molecule modified by a bifunctional chelator with a ^{99m}Tc precursor in aqueous environment. We are developing such a practical method for ^{18}F-labeling of targeting molecules based on silicon-fluoride chemistry. We have previously demonstrated the feasibility of this approach synthesizing a prototype of thiol-reactive ^{18}F-labeling agents (J. Labell. Comp. Radiopharm. 1999, 42, S1-S3). Thus, the maleimide-derivatised silanol (Si-OH) was converted into the corresponding Si-^{18}F by mixing Si-OH with cyclotron-produced aqueous [^{18}F]fluoride in the presence of HI. We now expand this approach to include amine-reactive ^{18}F-labeling agents, namely the Si-^{18}F derivatives (1 and 2) containing an aromatic aldehyde or N-hydroxysuccinimidyl ester group, respectively. Optimization of [^{18}F]fluorination as well as in vivo stability of the Si-^{18}F bond, using a normal mouse with microPET, are currently under investigation.



1 R = H
2 R = N-hydroxysuccinimidyl

Radiopharmaceutical Chemistry Track: Radiopharmacy Posters

Hall 2

No. 1099

HPLC PROTOCOLS FOR ROUTINE TC RADIOPHARMACEUTICAL QUALITY CONTROL. J. B. Slater*, J. W. Gunn, and P. S. Assaad, Loma Linda University, Loma Linda, CA. (101383)

Objectives: We have developed a cost-efficient method to ensure quality control of radiopharmaceuticals on a daily basis. Typically the integrity of Tc-labeled drugs is affirmed using ITLC. However; due to its accuracy and reproducibility, HPLC is favored over paper chromatography. Solvent protocols adequate for assessing the purity of five Tc radiopharmaceuticals were found using HPLC. **Methods:** A Waters HPLC system was used with their Millenium^{32} software. The system included the Waters 600 pump, 996 UV array dectector, 717 injector system and two bioscan radiation dectectors. The four solvents used were: (A) 0.05M NH_4SO4:MeOH (35:65) (B) 0.01M PO_4 buffer (pH 6.5):ethanol (95:5) (C) C^3HCN (D) 0.01M PO_4 buffer (pH 7.0):C^3HCN:MeOH (48:50:2) The solvents were used in the following manner: Cardiolite® (100%A), MAG_3® (100%B), Ceretec® (100%D), Choletec® (65%B & 35%C) HDP® (40%B & 60%C gradient to 100%B). Each run on HPLC consisted of two injections, each with a duration of six minutes. The first injection was used to equilibrate the system, while the second run was used to evaluate the pharmacological purity of each kit. **Results:** The technetium bound to the kit was separated from the unbound technetium by an average of three minutes. The unbound technetium was found to have a retention time of approximately 1-2 minutes, while the bound technetium appeared at 4-5 minutes. Using techniques provided by the software each of these two peaks can be integrated to precisely analyze the quality of the labeled drug. **Conclusion:** Using HPLC, we were able to determine solvent systems to analyze the purity of five commonly used Tc radiopharmaceuticals. This method is user friendly since it allows one to test these radiopharmaceuticals in any combination or order. This makes HPLC a practical approach for routine quality control of Tc radiopharmaceuticals.

No. 1100

EFFECT OF CHEMOTHERAPEUTIC DRUGS ON THE BIODISTRIBUTION OF A RADIOPHARMACEUTICAL USED FOR RENAL EVALUATIONS IN BALB/C FEMALE MICE. M. Bernardo-Filho*, D. M. Mattos, M. L. Gomes, R. S. Freitas, E. F. Paula, E. M. Boasquevisque, and V. N. Cardoso, Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil; Instituto do Câncer (INCA), Rio de Janeiro, Brazil; Universidade Federal de Minas Gerias (UFMG), Belo Horizonte, Brazil. (100080)

The biodistribution of radiopharmaceuticals can be altered by drugs. Knowledge of such altered biodistribution is important both in making diagnostic inferences and in dosimetric considerations. Vincristine and mitomycin-C are used in chemotherapeutic regimens. The biological activities of vincristine can be explained by its ability to bind to tubulin and to block the capability of the protein to polymerize into microtubules. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death. Mitomycin-C becomes a bifunctional or trifunctional alkylating agent. This drug inhibits deoxyribonucleic acid

high levels of EGFR. PEG-modification of C225 markedly reduced its liver uptake, resulting in improved visualization of EGFR-positive tumors. Using PEG as a linker between the monoclonal antibody and metal chelator is a useful strategy to optimize the imaging characteristics of antibody-based scintigraphic agents.

No. 1075

SYNTHESIS OF A NEW MALEIMIDE-CONTAINING THIOL-REACTIVE ^{18}F-LABELING AGENT, *N*-{4-[(4-^{18}FLUOROBENZYLIDNE)AMINOOXY] BUTYL}MALEIMIDE, AND ITS APPLICATION TO THE LABELING OF AN OLIGODEOXYNUCLEOTIDE. T. Toyokuni*, J. C. Walsh, R. J. Abdel-Jalil, A. Dominguez, J. E. Shively, N. Satyamurthy, J. R. Barrio, M. E. Phelps, A. M. Wu, S. S. Gambhir, UCLA School of Medicine, Los Angeles, CA; Beckman Research Institute of the City of Hope, Duarte, CA. (201061)

Objectives: Advances in biotechnology have lead to an appreciation of molecules that selectively target cancer at the cellular level. Examples include antisense oligodeoxynucleotides (ODNs) to oncogene mRNAs, engineered antibody fragments to tumor-associated antigens, and peptide ligands for tumor cell-surface receptors. We are interested in the development of simple and general ^{18}F-labeling agents to convert these targeting molecules into target-specific imaging probes for PET. We report the synthesis of a maleimide-containing thiol-reactive ^{18}F-labeling agent (1). **Methods:** The key synthetic step involves the utilization of aldehyde–aminooxy coupling reaction between 4-^{18}Ffluorobenzaldehyde (2) and a new bifunctional linker, *N*-[4-(aminooxy)butyl]maleimide (3). The new linker 3 was readily synthesized from either 1, 4-butanediol or tetrahydrofuran. **Results:** The coupling of 2 and 3 proceeded efficiently at room temperature for 15 min. Thus, 1 was synthesized in ~35% radiochemical yield (decay corrected) within ~60 min from EOB. The specific activity was ~3000 Ci/mmol at EOS. The 5′-thiol-functionalized anti-*c-myc* ODN, HS-$(CH_2)_6PO_4$-AACGTTGAGGGGCAT, was radiolabeled using 1. Treatment of the ODN with 1 (~30 mCi) in PBS (pH 7.5) at room temperature for 10 min afforded, after purification and reconstitution, chemically and radiochemically pure ^{18}F-labeled ODN (~1mCi) in PBS (1 mL). **Conclusion:** The maleimide-containing thiol-reactive ^{18}F-labeling agent 1 is now available for efficient radiolabeling of ODNs. Application of 1 for ^{18}F-labeling of antibody fragments is underway.

No. 1076

A-RING FLUORINATED 16α-^{18}FFLUOROESTRADIOLS AND THEIR 11β-METHOXY DERIVATIVES FOR ESTROGEN RECEPTOR IMAGING OF BREAST CANCER. Y. Seimbille*, J. Rousseau, H. Ali, F. Bénard, J. E. Van Lier, Université de Sherbrooke, Sherbrooke, PQ, Canada; Université de Sherbrooke, Sherbrooke, QC, Canada. (201417)

Background: The determination of estrogen receptor (ER) levels in breast tumors plays a crucial role in the choice of an appropriate therapy, and provides important prognostic information. ER concentrations in breast tumors are routinely determined via a biochemical assay of biopsy samples. This procedure is invasive and may introduce sampling heterogeneity problems. In vivo imaging of ER via SPECT or PET analysis provides a non-invasive alternative procedure. Radiolabeled estrogens that have been used in the clinic for PET imaging include 16α-^{18}Ffluoroestradiol (FES). However FES is readily metabolized preventing optimal localization at the ER-binding sites. **Objectives:** To develop an improved PET-scanning agent for ER we sought to increase the metabolic stability of FES via the addition of a F-atom at either the 2- or 4-position, with and without an added 11β-methoxy group. **Methods:** The 3-methoxymethyl ether of 2- or 4-fluoro-3, 16β, 17β-epiestriol, or their 11β-methoxy derivatives, were converted to the reactive 16β, 17β-cyclic sulfate intermediates. The latter were stereoselectively opened via a nucleophilic fluorination with ^{18}F-fluoride, followed by rapid hydrolysis of the protecting ether and sulfate groups in ethanolic-acid solution. The final 2, 16α- or 4, 16α-^{18}Fdifluoroestradiol-17β and their 11β-methoxy analogs were purified by HPLC and iv administered to immature female rats to establish biodistribution pattern relative to FES. **Results:** The identity of the new radiopharmaceuticals was confirmed by comparing their HPLC mobility with that of the cold analogs. Biodistribution in immature female rats revealed that the 11β-methoxy-4, 16α-^{18}Fdifluoroestradiol showed the highest receptor-mediated uterus uptake values. **Conclusion:** These data suggest that the addition of both a 4-fluoro and 11β-methoxy group onto FES may provide an improved radiopharmaceutical for PET imaging of estrogen receptor densities in breast cancer patients.

No. 1077

SYNTHESIS OF ^{18}F-FLUOROPROPYLSQUALAMINE AS ANGIOGENESIS IMAGING AGENT. C.-Y. Shiue*, G. G. Shiue, A. A. Alavi, S. Jones*, M. A. Zasloff*, University of Pennsylvania, Philadelphia, PA; Magainin Pharmaceuticals. Inc.*, Plymouth Meeting, PA. (202459)

Angiogenesis is an essential event in many physiological processes such as wound repair, ovulation, and embryogenesis. Neovascularization is also a key component of many pathological processes such as inflammation, glaucoma, diabetic, myocardial ischemia, psoriasis and tumor formation. Recently, we have shown that squalamine (1) inhibits angiogenesis and solid tumor growth *in vivo* and perturbs embryonic vasculature, and that N-fluoropropylsqualamine (2) has similar biological activities as that of squalamine. We have synthesized F-18 labeled compound 2 and intend to evaluate it as angiogenesis imaging agent. **Methods:** Compound 2 was synthesized in two steps. Nucleophilic substitution of 1, 3-propyldiol-di-p-tosylate with $K^{18}F$ in CH_3CN at 90°C for 20 min gave ^{18}F-fluoropropyl tosylate. Alkylation of squalamine (free base) with ^{18}F-fluoropropyl tosylate in DMF at 110°C for 20 min followed by purifications with Silica Sep-Pak (CH_2Cl_2:CH_3OH, 8:2, 30 mL and discarded). The crude product was rinsed out with CH_2Cl_2:CH_3OH:NH_4OH, 6:3:1, 6 mL) and purified further with HPLC (Phenomenex, Luna 2, Silica, 4.6 × 250 mm, CH_3CN:H_2O:TFA, 85:15:0.1%, 1mL/min). **Results:** Fluorine-18 labeled N-fluoropropylsqualamine (2) was synthesized in 4–7% yield in a synthesis time of 100 min from EOB. **Conclusion:** Compound 2 can be prepared in two steps. The one-step synthesis of Compound 2 from N-iodopropyl and N-tosylpropyl squalamine, and the evaluation of F-18 labeled compound 2 as angiogenesis imaging agent are continuing.

No. 1078

SYNTHESIS OF 4-[(4-^{18}FFLUOROBENZYLIDENE) AMINOOXY]BUTYL VINYL SULFONE AS AN AMINE- AND THIOL-REACTIVE ^{18}F-LABELING AGENT. R. J. Abdel-Jalil*, J. C. Walsh, J. L. Stone, J. E. Shively, N. Satyamurthy, J. R. Barrio, M. E. Phelps, A. M. Wu, S. S. Gambhir, T. Toyokuni, Crump Institute for Molecular Imaging, UCLA School of Medicine, Los Angeles, CA; Beckman Research Institute of the City of Hope, Duarte, CA. (201851)

Objectives: We are developing simple and general methods to transform targeting molecules into target-specific cancer imaging probes for PET. Particularly, ^{18}F-labeled oligodeoxynucleotides (ODNs), monoclonal antibody fragments and peptide ligands for cell-surface receptors have the potential to become versatile probes for imaging oncogenes and their protein products. We report the synthesis of a vinylsulfone-containing ^{18}F-labeling agent 1, designed to label targeting molecules either through a thiol group (at pH 7–8) or an amino group (at pH 8). **Methods:** The synthesis of 1 utilizes efficient aldehyde–aminooxy coupling reaction of 4-^{18}Ffluorobenzaldehyde with a new bifunctional linker, 4-(aminooxy)butyl vinyl sulfone (2). The bifunctional linker 2 was synthesized from 1, 4-dibromobutane via a sequence of reactions including selective aminooxidation, formation of a thioether, oxidation to a sulfone and β-elimination to a vinylsulfone. **Results:** 4-^{18}FFluorobenzaldehyde was reacted with 2 at room temperature for 15 min to yield 1 in ~30% radiochemical yield (decay corrected) based on ^{18}Ffluoride within ~60 min from EOB. **Conclusion:** The vinylsulfone-containing thiol-reactive ^{18}F-labeling agent 1 has been synthesized. Application of 1 for labeling monoclonal antibody fragments and ODNs are currently underway.

No. 1079

IMPROVED SYNTHESIS, PURIFICATION AND CHARACTERIZATION OF ^{18}F3-FLUORO-L-α-METHYLTYROSINE. N. Vasdev*, R. Chirakal, G. J. Schrobilgen, C. Nahmias, McMaster University, Hamilton, ON, Canada; Hamilton Health Sciences Corp., Hamilton, ON, Canada. (200729)

Objectives: Fluorine-18 labelled 3-fluoro-L-α-methyltyrosine (3-F-α-MT) has shown to be a promising tumor imaging agent with PET. The syntheses of ^{18}F2- and 3-F-α-MT have been reported in 20.3 ± 5.1%

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